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L18: Entry 12 of 13 File: DWPI Jul 5, 1999

DERWENT-ACC-NO: 1989-078826
DERWENT-WEEK: 199932

TITLE: Purificn. of biological fluids - by filtration through ultrafiltration membrane

INVENTOR: ESHKOL, A; MAILLARD, F ; STILES, G E

PATENT-ASSIGNEE:

ASSIGNEE	CODE
ARES SERONO NV	ISTF
ARES TRADING SA	ISTF

PRIORITY-DATA: 1987US-0096561 (September 11, 1987)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 2914666 B2	July 5, 1999		016	A61K009/08
EP 307373 A	March 15, 1989	E	016	
AU 8821408 A	March 16, 1989		000	
JP 02000102 A	January 5, 1990		000	
EP 307373 B1	November 18, 1993	E	022	C07K003/26
DE 3885686 G	December 23, 1993		000	C07K003/26
ES 2047043 T3	February 16, 1994		000	C07K003/26
CA 1328625 C	April 19, 1994		000	B01D061/14

DESIGNATED-STATES: AT BE CH DE ES FR GB GR IT LI LU NL SE AT BE CH DE ES FR GB GR IT LI LU NL SE

CITED-DOCUMENTS:A3...9026; EP 209331 ; EP 219295 ; GB 2134528 ; No-SR.Pub ; 3.Jnl.Ref

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
JP 2914666B2	September 10, 1988	1988JP-0225563	
JP 2914666B2		JP 2000102	Previous Publ.
EP 307373A	September 9, 1988	1988EP-0830362	
JP02000102A	September 10, 1988	1988JP-0225563	
EP 307373B1	September 9, 1988	1988EP-0830362	
DE 3885686G	September 9, 1988	1988DE-3885686	
DE 3885686G	September 9, 1988	1988EP-0830362	
DE 3885686G		EP 307373	Based on
ES 2047043T3	September 9, 1988	1988EP-0830362	
ES 2047043T3		EP 307373	Based on
CA 1328625C	September 9, 1988	1988CA-0576936	

INT-CL (IPC): A61K 9/08; A61K 35/12; A61K 35/30; A61K 37/36; A61K 38/27; A61M 1/36; B01D 13/00; B01D 61/14; B01D 69/02; C07K 3/26

ABSTRACTED-PUB-NO: EP 307373A
BASIC-ABSTRACT:

Biological materials or prods. derived from them are purified by filtration through a membrane with a molecular-wt. cut-off of 100,000.

A 'Millipore PTHK' polysulphone membrane, which retains at least 99% blue dextran and at least 95% IgG and passes at least 60% albumin, is used. The filtrate may be treated with urea to inactivate any residual viral infectivity. The urea may then be removed by ultrafiltrati on.

USE/ADVANTAGE - The process is useful for removing viruses from biological fluids, esp. for removing retroviruses and 'slow' viruses (e.g. HIV, scrapie or CJD virus) from hormone-contg. extracts, e.g. pituitary extracts contg. human growth hormone (LGH). The process is simpler and less expensive than chemical and/or thermal methods, and the treated fluids are ready for immediate use.
ABSTRACTED-PUB-NO:

EP 307373B
EQUIVALENT-ABSTRACTS:

A process for substantially reducing the viral infectivity of or removing viral contaminants from a biological product without significant loss thereof characterised in that it comprises the tangential flow filtration of a fluid containing the biological product through an ultrafiltration membrane having a 100,000 dalton cut-off and the recovery of the biological product form the filtrate.

CHOSEN-DRAWING: Dwg.0/0 Dwg.0/0

TITLE-TERMS: PURIFICATION BIOLOGICAL FLUID FILTER THROUGH ULTRAFILTER MEMBRANE

DERWENT-CLASS: A89 B04 D22 J01 P34

CPI-CODES: A12-V03C1; A12-V03D; A12-W11A; B04-B02B4; B04-B02D4; B11-B; B12-A06; D09-A02; J01-C03; J01-F02;

CHEMICAL-CODES:

Chemical Indexing M1 *01*
Fragmentation Code
M423 M424 M740 M750 M903 N161 Q261 Q431 V500 V560
Registry Numbers
1704X 1724X 1711X 1714X

Chemical Indexing M1 *02*
Fragmentation Code
M423 M424 M720 M740 M903 N164 P625 Q261 Q431 V624
Registry Numbers
1704X 1724X 1711X 1714X

Chemical Indexing M1 *03*
Fragmentation Code
C316 K0 K4 K442 K499 M280 M312 M313 M314 M315
M316 M323 M331 M332 M333 M342 M383 M393 M423 M424
M510 M520 M530 M540 M740 M781 M903 N161 N164 Q261
Q431 Q508 V743
Registry Numbers
1704X 1724X 1711X 1714X

POLYMER-MULTIPUNCH-CODES-AND-KEY-SERIALS:
Key Serials: 0231 1309 3270 2769 3256

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SECONDARY-ACC-NO:
CPI Secondary Accession Numbers: C1989-035012

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End of Result Set

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L5: Entry 1 of 1 File: USPT Mar 20, 1990

DOCUMENT-IDENTIFIER: US 4909941 A
TITLE: High performance liquid chromatography mobile phase

DRTL:

FIG. 1: COLUMN: Partisil 10 ODS (300 .times. 4 mm) Buffers (A) 0.1% citric acid in H.sub.2 O/2-Propanol (9:1) (B) 0.1% citric acid in H.sub.2 O/2-Propanol (1:9) Linear gradient from 0 to 100% in 1 hour at 1 ml/min. Detector: Waters Model 450 variable wave length detector at 220 nm. A 2.0 CHART: 200 mm/hr Insulin (porcine) retention time = 17-18 min. Albumin (bovine) retention time = 25-27 min. FIG. 2 COLUMN: Rad-Pak = C.sub.18 Buffers: (As in FIG. 1) Linear gradient from 0 to 100% in 1 hour at ml/min. Detention: 280 nm A 0.1 Chart: 200 mm/hr Albumin (bovine) .beta.-lactoglobulin (A) .beta.-lactoglobulin (B) not separated FIG. 3a Instrument: Prep/LC-500 (Waters) Column: Prep-Pak-C.sub.18 (1 cartridge) Buffers: (A) 0.1% 1% citric acid H.sub.2 O/95% EtOH (9:1) 3 liters (B) 0.1% citric acid in H.sub.2 O/95% EtOH (1:9) 3 liters Gradient concave at 50 ml/min. Detection: 280 nm; A 1.0 and 2.0 (see chart) CHART: 200 mm/hr 1.0 g of insulin (porcine; Nordisk) retention time 65 min. FIG. 3b: Insulin after chromatography on the Prep/LC-500 (see 3a) Column: Rad-Pak-C.sub.18 Buffers: (A) 0.1% citric acid in H.sub.2 O/2-Propanol (9:1) (B) 0.1% citric acid in H.sub.2 O/2-Propanol (1:9) Gradient: linear from 0 to 100% at 1 ml/min. in 1 hour Detection: 220 nm A 2.0 125 .mu.g of insulin. Retention time 23 min. FIG. 4: Instrument: Prep/LC-500 Waters. 1.0 g bovine serum albumin (BSA) COLUMN: Prep-Pak-C.sub.18 (1 cartridge) Buffers: (A) 0.1% citric acid in H.sub.2 O/95% EtOH (9:1) 2 liters (B) 0.1% citric acid in H.sub.2 O/95% EtOH (1:9) 3 liters Gradient: concave at 50 ml/min Detection: 280 nm A 2.0 Chart: 200 mm/hr Retention time 85 min (top of peak) FIG. 5: COLUMN: Rad-Pak-C.sub.18 Buffers: (A) 0.1% citric acid in H.sub.2 O/2-Propanol (9:1) (B) 0.1% citric acid in H.sub.2 O/2-Propanol (1:9) Detection: 220 nm Insulin (porcine: Nordisk) 50 .mu.g Albumin (bovine: Sigma) 50 .mu.g .beta.-Lactoglobulin A 50 .mu.g .beta.-Lactoglobulin B 50 .mu.g Detection: 280 nm A 2.0 Chart: 200 mm/hr FIG. 6: Conditions as in FIG. 5 .beta.-Lactoglobulin A 50 .mu.g .beta.-Lactoglobulin B 50 .mu.g Detection: 280 nm A 2.0 Chart: 100 mm/hr Gradient: Linear from 25 to 50% in 1 hour at 0.5 ml/min. FIG. 7: Conditions as in FIG. 6 100 .mu.g of whey protein FIG. 8a: The separation of human growth hormone, Nordisk Insulin Laboratories. Conditions as in FIG. 5. GRADIENT: Linear 25 to 100% in 1.5 hr. at 0.5 ml/min. FIG. 8b: The separation of human growth hormone, New Zealand National Hormone Laboratory. Conditions as in FIG. 5 GRADIENT: Linear 25 to 50% in 1 hr. at 0.5 ml/min. FIG. 9: Coating of the column with acid. Column: Waters; 8 MBC1810 .mu., P4194A01 Solvent: water/2-propanol 9:1 Injections (repeated) of 1% (v/v) citric acid. (20 .mu.1) Detection: Differential Refractometer R401 Sensitivity X 2. FIG. 10: Column: Waters; 8NVC18 5.mu., P4136DO1 Solvent: water/2-propanol 9:1 Injection of 1% citric acid (20 .mu.l) Detection: R 401 sensitivity X1 FIG. 11: Shows a test mixture of proteins on 8 different columns. COLUMN A: SUPELCO: LC-3DP COLUMN B: WATERS: 8MBC1810 .mu. COLUMN C: SUPELCO: LC-318 COLUMN D: WATERS: NOVPAK-C18 COLUMN E: WATERS: 8NVC185 .mu. COLUMN F: VYDAC: PROTEIN-C4 COLUMN G: WHATMAN: PROTESIL-300 OCTYL-25 COLUMN H: SYNCHROM. INC.: SYNCHROPAK RP-P Buffer: citric acid/salt system Program: Linear gradient usually from 15-100% B in 1 hour at 1 ml/min. Sample: peak 1: insulin (porcine) peak 2: cytochrome C. (horse heart) peak 3: bovine serum albumin peak 4: .alpha.-lactalbumin peak 5: (+8): myoglobin (equine skeletal muscle) peak 6: .beta.-lactoglobulin B peak 7: .beta.-lactoglobulin A FIGS. 12A-12D: Separation of whey proteins. Mixture containing: peak 4: .alpha.-lactalbumin 6: .beta.-lactoglobulin B 7:

.beta.-lactoglobulin A Columns: A VYDAC: C4 B SUPELCO: LC-318 C SUPELCO: LC-3DP D SYNCHROM. INC.: SYNCHROPAK RP-P Buffer: citric acid system Program: linear gradient from 25-50% B in 1 hour at 1 ml/min. FIGS. 13A-13D Separation of insulins. Buffer: citric acid system Program: linear gradient from 13-20% B (column A) linear gradient from 15-25% B (others) in 1 hour at 1 ml/min. Sample: peak (A) equine insulin (B) bovine insulin (C) porcine insulin Columns used: A SUPELCO: LC-3DP B SUPELCO: LC-318 C VYDAC: PROTEIN-C.sub.4 D PROTESIL 300 OCTYL-25 FIG. 14: Preparative chromatography of 1.0 g of bovine serum albumin. Instrument: Prep-500 Waters Column: 1 C18 - cartridge Buffers: A: 0.1% citric acid + 1% NaCl in H.sub.2 O/95% EtOH 3:1 Buffer B: 0.1% citric acid + 1% NaCl in H.sub.2 O/95% EtOH 45:55 Gradient was concave and was made up from 1 liter buffer A and 5 1 liter buffer B. FIGS. 15 (a-c) On the Supelco LC-318 column was injected the general test mixture described before. Buffers: FIG. 15a: A: 0.1% citric acid + 1% salt in H.sub.2 O/IPA 9:1 B: 0.1% citric acid + 1%

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salt in H.sub.2 O/IPA 1:4 FIG. 15b: A: 0.1% v/v H.sub.3 PO.sub.4 + 1% salt in H.sub.2 O/IPA 9:1 B: 0.1% v/v H.sub.3 PO.sub.4 + 1% salt in H.sub.2 O/IPA 1:4 FIG. 15c: A: 0.1% TFA + 1% salt in H.sub.2 O/IPA 9:1 B: 0.1% TFA + 1% salt in H.sub.2 O/IPA 1:4 Linear gradients from 15-60% in 1 hour at 1 ml/min. for A and B From 5-60% B in 1 hour ml/min. for C. FIGS. 16A-16c On the Supelco LC-318 column was injected a mixture of: (a) equine insulin (b) bovine insulin (c) porcine insulin Buffers as in FIG. 15a Gradients: A and B from 15-25% B in 1 hour; 1 ml/min. C from 10-25% B in 1 hour; 1 ml/min. FIG. 17: On a Synchroprep column was injected: A. Human Serum Albumin (HSA) B. Bovine Serum Albumin (BSA) C. Porcine Insulin. Other parameters are set out in Example 11 FIG. 18: On the columns listed a general protein mixture was injected. Other parameters are set out in Example 12. FIG. 19: As in FIG. 18 except insulins were injected as exemplified in Example 12. FIG. 20: As in FIG. 18 except whey proteins were injected as exemplified in Example 12. FIG. 21: Column Synchropak RP-P Sample: 5 mg of rhGH Buffers: System 1: Buffer A: 0.1% TFA in H.sub.2 O/CH.sub.3 CN 9:1 Buffer B: 0.1% TFA in H.sub.2 O/CH.sub.3 CN 1:4 System 2: Buffer A: 0.1% H.sub.3 PO.sub.4 + 1% NaCl in H.sub.2 O/CH.sub.3 CN 9:1 Buffer B: 0.1% H.sub.3 PO.sub.4 + 1% NaCl in H.sub.2 O/CH.sub.3 CN 1:4 System 3: Buffer A: 0.1% citric acid + 1% guanidine.HCl in H.sub.2 O/CH.sub.3 CN 9:1 Buffer B 0.1% citric acid + 1% guanidine.HCl in H.sub.2 O/CH.sub.3 CN 1:4 FIG. 22: Column: As in FIG. 21 Sample: 5 mg of met.hGH Buffers: As in FIG. 21 FIG. 23: Column: As in FIG. 21 Sample: 2 ml of recombinant human insulin Buffers: As in FIG. 21, system 4.

DEPR:
In examples 1 and 2, insulin and serum albumin were examined as examples of small and large proteins respectively. The separation of albumin was found to be much more demanding than insulin. Only the superior eluotrophic properties of the ionic modifiers of the formula YCOOH allowed good recovery and separation of albumin in the presence of ethanol-water mixtures. Example 8 shows that the nature of the gradient of organic solvent is important.

DEPR:
(A) 0.1% citric acid + 1% sodium chloride in water/95%-ethanol 9:1.

DEPR:
(B) 0.1% citric acid + 1% sodium chloride in water/95%-ethanol 1:4.

DETL:
TABLE 1 ______ pH of Elution Acids pKa.sub.1 Buffer A of Albumin Remarks ______ Hydrochloric + Sulphuric + strong acids damaging to C.sub.18 -column TFA 0.30 2.15 Oxalic 1.23 2.23 + Maleic 1.83 2.38 - Phosphoric 2.12 2.57 + Malonic 2.83 2.57 ++ Tartaric* 2.98 2.75 + Peak shape bad Glucuronic 3.00 2.75 + Fumaric 3.03 2.75 - Lactic 3.08 2.86 + Peak shape bad Citric 3.14 3.00 ++ Galacturonic .about. 3.5 3.30 ++ Formic 3.75 3.30 ++ Glycolic 3.83 3.14 + Peak shape bad Ascorbic 4.10 3.40 - Succinic 4.16 5.10 + Less then 0.1% used because of poor solu- bility. Peak shape bad Acetic 4.75 3.40 + Peak shape bad ______ *TARTARIC ACID MUST NOT BE USED WITH NaCl

as there is a strong tendency for sodium hydrogen tartrate to crystallise on column, in pumps and samples etc.

DETL:
TABLE 2 ____________________________________ pH of Elution of Acids PKa.sub.1 Buffer A Insulin Remarks ____________________________________ Hydrochloric + Sulphuric + strong acids damaging to C.sub.18 -column TFA 2.15 + Oxalic 1.23 2.23 + Maleic 1.83 2.38 - Phosphoric 2.12 2.57 + Malonic 2.83 2.57 ++- Tartaric* 2.98 2.75 + Peak shape bad Glucuronic 3.00 2.75 + Fumaric 3.03 2.75 - Lactic 3.08 2.86 + Peak shape bad Citric 3.14 3.00 ++ Galacturonic .about. 3.5 3.30 ++ Formic 3.75 3.30 ++ Glycolic 3.83 3.14 + Ascorbic 4.10 3.40 - Succinic* 4.16 5.30 + *Saturated solution <0.1% Peak shape bad Acetic 4.75 3.40 + Peak shape bad ____________________________________ *TARTARIC ACID MUST NOT BE USED WITH NaCl as there is a strong tendency for sodium hydrogen tartrate to crystallise on column, in pumps and samples etc.

DETL:
TABLE 5 ____________________________________ SALTS REMARKS ____________________________________ sodium chloride good potassium chloride good ammonium chloride good lithium chloride moderate ammonium sulphate unsatisfactory sodium sulphate unsatisfactory guanidine hydrochloride good ____________________________________

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L7: Entry 1 of 1 File: USPT Mar 20, 1990

DOCUMENT-IDENTIFIER: US 4909941 A
TITLE: High performance liquid chromatography mobile phase

DEPR:
In examples 1 and 2, insulin and serum albumin were examined as examples of small and large proteins respectively. The separation of albumin was found to be much more demanding than insulin. Only the superior eluotrophic properties of the ionic modifiers of the formula YCOOH allowed good recovery and separation of albumin in the presence of ethanol-water mixtures. Example 8 shows that the nature of the gradient of organic solvent is important.

DEPR:
Instead of salts, neutral, non-ionic compounds were added to the mobile phase. For example, urea, glucose and glycerol were tried but did not produce useful separations.

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L9: Entry 28 of 28 File: DWPI Mar 15, 1989

DERWENT-ACC-NO: 1989-078293
DERWENT-WEEK: 198911

TITLE: Sterilisation of plasma contg. blood coagulation factor VIII - by treating with tri-n-butyl phosphate and sodium cholate or Tween-800 and beta-propiolactone or UV irradiation

ABTX:
Pr ef. sterilisation is carried out at +4 to 37 deg.C (esp. 23 deg.C), at pH 4.5-8.3 (esp. 7.2). Pref. the tri-n-butylphosphate is used in a concn. of 0.05-0.5% (esp. 0.3%) and the sodium chlolate in a concn. of 0.03-0.4% (esp. 0.2%). Alternatively pref. tri-n-butylphosphat e is used in a concn. of 0.3% and the tween 80 in a concn. of 0.15-2%. Pref. the B-butyrolactone is used in a concn of 0.01-0,25% (pref. 0.05%). Pref. the UV light used is 254 nm.

ABTX:
USE/ADVANTAGE - The process gives better yields of factor VIII activity while inactivating lipid viruses as well as protein viruses pathogenic to humans. The effect of the two treatments is synergistic.

ABEQ:
Pref. sterilisation is carried out at +4 to 37 deg.C (esp. 23 deg.C), at pH 4.5-8.3 (esp. 7.2). Pref. the tri-n-butylphosphate is used in a concn. of 0.05-0.5% (esp. 0.3%) and the sodium chlolate in a concn. of 0.03-0.4% (esp. 0.2%). Alternatively pref. tri-n-butylphosphat e is used in a concn. of 0.3% and the tween 80 in a concn. of 0.15-2%. Pref. the B-butyrolactone is used in a concn of 0.01-0,25% (pref. 0.05%). Pref. the UV light used is 254 nm.

ABEQ:
USE/ADVANTAGE - The process gives better yields of factor VIII activity while inactivating lipid viruses as well as protein viruses pathogenic to humans. The effect of the two treatments is synergistic.

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#64

L9: Entry 6 of 28 File: USPT Aug 19, 1997

US-PAT-NO: 5659017
DOCUMENT-IDENTIFIER: US 5659017 A

TITLE: Anion exchange process for the purification of Factor VIII

DATE-ISSUED: August 19, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bhattacharya; Prabir	Walnut	CA		
Motokubota; Toshiharu	Arcadia	CA		
Fedalizo; Norman M.	Rowland Heights	CA		

US-CL-CURRENT: 530/383

CLAIMS:

What is claimed is:

1. A process for separating Factor VIII complex (a complex of Factor VIII:C and vWF) from an impure protein fraction containing Factor VIII complex, the process comprising the steps of:
(a) providing an aqueous solution of an impure protein fraction containing Factor VIII complex;
(b) applying the impure protein fraction solution to an anion exchange chromatographic medium to thereby bind Factor VIII complex to the anion exchange medium;
(c) eluting Factor VIII complex from the anion exchange medium using an eluting buffer comprising from about 0.10M to about 0.20M CaCl.sub.2 ; and
(d) recovering Factor VIII complex eluted from the column.
2. The process of claim 1 wherein the CaCl.sub.2 present in the eluting buffer is at a concentration ranging from about 0.14M to about 0.2M.
3. The process of claim 2 wherein the CaCl.sub.2 present in the eluting buffer is at a concentration of 0.2M.
4. The process of claim 1 wherein the eluting buffer further comprises histidine at a concentration of from about 0.015M to about 0.035M.
5. The process of claim 4 wherein the concentration of histidine in the eluting buffer is 0.025M.
6. The process of claim 1 wherein the eluting buffer has a pH from about 6.0 to about 8.0.
7. The process of claim 6 wherein the pH of the eluting buffer is maintained at about 6.8.
8. The process of claim 1 wherein the anion exchange chromatography medium comprises a quaternary amino ethyl resin.
9. The process of claim 8 wherein the resin is a QAE-550C resin manufactured by TosoHaas.
10. The process of claim 1 wherein the aqueous solution of an impure protein fraction containing Factor VIII complex is derived from cryoprecipitate.
11. A process for separating Factor VIII complex (a complex of Factor VIII:C and vWF) from an impure protein fraction containing Factor VIII complex, the process comprising the steps of:
(a) providing an aqueous solution of an impure protein fraction containing Factor VIII complex;
(b) applying the impure protein fraction solution to an anion exchange chromatographic medium located in a column to thereby bind Factor VIII complex to

the anion exchange medium;
(c) eluting Factor VIII complex from the anion exchange medium using an eluting buffer comprising from about 0.10M to about 0.20M CaCl.sub.2 ;
(d) recovering Factor VIII complex eluted from the column;
(e) adding glycine and NaCl to the recovered eluate to thereby precipitate Factor VIII complex; and
(f) recovering Factor VIII precipitated complex.
12. The process of claim 11 wherein the CaCl.sub.2 present in the eluting buffer is at a concentration ranging from about 0.14M to about 0.2M.
13. The process of claim 12 wherein the CaCl.sub.2 present in the eluting buffer is at a concentration of 0.2M.
14. The process of claim 11 wherein the eluting buffer further comprises histidine at a concentration of from about 0.015M to about 0.035M.
15. The process of claim 14 wherein the concentration of histidine in the eluting buffer is 0.025M.
16. The process of claim 11 wherein the eluting buffer has a pH from about 6.0 to about 8.0.
17. The process of claim 16 wherein the pH of the eluting buffer is maintained at about 6.8.
18. The process of claim 11 wherein the anion exchange chromatography medium comprises a quaternary amino ethyl resin.
19. The process of claim 18 wherein the resin is a QAE-550C resin provided by TosoHaas.
20. The process of claim 11 wherein the aqueous solution of an impure protein fraction containing Factor VIII complex is derived from cryoprecipitate.
21. The process of claim 11 wherein the Factor VIII complex is precipitated with about 1.5M to about 2.5M glycine and about 1.2M to about 3.0M NaCl.
22. The process of claim 21 wherein the Factor VIII complex is precipitated with about 2M glycine and about 1.6M NaCl.
23. A process for separating Factor VIII complex (a complex of Factor VIII:C and vWF) from cryoprecipitate, comprising the steps of:
(a) dissolving the cryoprecipitate in a heparin solution;
(b) adding polyethylene glycol to the dissolved cryoprecipitate to precipitate proteins other than Factor VIII complex;
(c) separating the polyethylene glycol supernatant from the precipitate;
(d) treating the recovered supernatant with an organic solvent and a detergent to deactivate viruses;
(e) applying the viral deactivated supernatant to an anion exchange chromatographic medium containing a quaternary amino ethyl group located in a column to thereby bind Factor VIII complex to the anion exchange medium;
(f) eluting Factor VIII complex from the anion exchange medium using an eluting buffer comprising from about 0.10M to about 0.20M CaCl.sub.2 and about 0.025M histidine at a pH of 6.8;
(g) recovering Factor VIII complex eluted from the column;
(h) adding glycine and NaCl to the recovered eluate to a final concentration of 2.0M glycine and 1.6M NaCl to thereby precipitate Factor VIII complex;
(i) recovering Factor VIII precipitated complex;
(j) reconstituting Factor VIII complex in a solution comprising 0.1M arginine, 0.025M histidine, 3% (wt/vol) cyclodextrin at a pH of 7.3;
(k) lyophilizing the reconstituted Factor VIII complex; and
(l) dry heating the lyophilized Factor VIII complex at 80.degree. C. for 72 hours.

D. 6

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reverse Step # 6 f

L9: Entry 14 of 28 File: USPT Feb 22, 1994

DOCUMENT-IDENTIFIER: US 5288853 A
TITLE: Factor viii purification process

DEPR:
In an exemplary embodiment of practice of this invention, the Factor VIII complex production process includes steps for inactivating viruses that may be present in such blood products, e.g., hepatitis B virus, hepatitis non-A/non-B virus, HIV (AIDS virus), Cytomegalovirus, Epstein-Barr virus, and the like, prior to the affinity chromatography step. In one embodiment, a solution comprising both an organic solvent and a detergent, is added to the PEG supernatant to inactivate virus that may be present. The amount of organic solvent and detergent added preferably results in a solution comprising about 0.3% (wt/vol) organic solvent and about 1% (wt/vol) detergent. A detergent useful in practice of principles of the invention is one sold by under the trademark "TWEEN-80" by Fisher Scientific, of Springfield, N.J.; another is a detergent sold under the trademark "TRITON X-100," by Aldrich Company, of Milwaukee, Wis. Useful organic solvents are tri-n-butyl-phosphate (TNBP), ethyl ether, and the like. The solution is incubated for about 6 hours to about 7 hours, at a temperature of from about 24.degree. C. to about 30.degree. C. Inactivation of virus using organic solvent/detergent mixture is described in U.S. Pat. No. 4,540,573, which issued on Sep. 10, 1985 to Neurath et al., and which is incorporated herein by this reference.

DEPR:
In an exemplary embodiment of the practice of this invention, Factor VIII complex solution from the viral inactivation step (the Factor VIII complex containing impure protein fraction) is applied to the chromatography column containing a heparin-coupled chromatographic medium by pouring the solution through the column. While the cross-linker agarose resin is preferred, other heparin or heparin sulfate-coupled media are also suitable for practice of this invention. Other dextran sulfate compounds coupled to a chromatography medium would also be useful in the purification process. The flow rate of the column is about 0.35 ml per min. for a small (about 5 ml) column, to about 2 ml per min. for a large (about 50 ml) column. As the impure protein fraction flows through the column, Factor VIII complex binds to the heparin ligand on the heparin-coupled chromatographic medium, while other proteins pass through the chromatographic medium in the column and flow from the column as effluent. Preferably, no more that about 20 units of Factor VIII:C activity are applied to the column per ml of heparin-coupled chromatography medium in the column when, as in one exemplary embodiment, 1000 units of heparin are bound per ml of activated resin. When greater than about 20 units of Factor VIII:C activity are added per ml of heparin-coupled chromatographic medium, the excess Factor VIII complex is not bound, but is instead washed through the column into the column effluent. If less than about 20 units of Factor VIII:C activity per ml are added, the maximum binding capacity of the heparin-coupled chromatographic medium (at 1000 units of heparin per ml of activated resin) is not being used.

DEPR:
In an exemplary embodiment of the practice of this invention, Factor VIII complex solution from the viral inactivation step (the Factor VIII complex containing impure protein fraction) is applied directly to the washed heparin-coupled chromatographic medium and mixed for about 30 min. to about 45 min. for batch processing. During this time, the Factor VIII complex binds to the heparin ligand on the chromatographic medium, leaving a supernatant

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containing proteins other than Factor VIII complex in solution. The chromatographic medium is removed by decanting the supernatant, and the medium is then washed to remove unbound proteins. In one exemplary embodiment, the washing is effected by resuspending the Factor VIII complex-bound, heparin-coupled chromatographic medium in about 5 to 10 volumes of a solution comprising about 0.015M to 0.035M buffer, such as histidine, pH 6.5 to 7.5, comprising a salt solution, such as LiCl, NaCl, or KCl, at a concentration of about 0.1M to about 0.15M. Preferably, the solution comprises 0.025M histidine at a pH of 6.8 comprising 0.15M NaCl. The Factor VIII complex-bound, heparin-coupled chromatographic medium is removed from the wash solution by decanting the supernatant, i.e., the wash solution. The Factor VIII complex remains bound to the heparin-coupled chromatographic medium throughout the wash procedure.

DEPR:
The PEG precipitate was separated from the Factor VIII complex-containing supernatant solution by centrifugation. The supernatant, i.e., the Factor VIII complex containing impure protein fraction, was recovered. The supernatant was then treated to inactivate viruses which may be present in the blood products, by adding a solution containing about 0.3% (wt/vol) tri-n-butylphosphate and about 1% (wt/vol) TWEEN-80, and incubating at 25.degree. C. for about 6 hrs.

DEPR:
The viral-inactivated supernatant solution, i.e., the viral-inactivated Factor VIII complex containing impure protein fraction, was clarified by filtration, and was then recovered for further purification of Factor VIII complex by affinity chromatography on a heparin-coupled chromatographic medium.

DEPR:
The PEG precipitate was separated from the Factor VIII complex-containing supernatant solution by centrifugation. The PEG supernatant, i.e., the Factor VIII complex containing impure protein fraction, was recovered. The supernatant was then treated to inactivate viruses which may be present in the blood products, by the addition of a solution containing about 0.3% (wt/vol) tri-n-butylphosphate and about 1% (wt/vol) TWEEN-80 and incubating at 25.degree. C. for about 6 hrs.

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The viral-inactivated supernatant solution, i.e., the viral-inactivated Factor VIII complex containing impure protein fraction, was clarified by filtration and then recovered for further purification of Factor VIII complex by affinity chromatography on a heparin-coupled chromatographic medium.

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USPT,JPAB,EPAB,DWPI	l11 and ((sodium chloride or NaCl or calcium chloride or CaCl$1 or salt$3) or (urea or rhodanide or guanidinium))	108	L12
USPT,JPAB,EPAB,DWPI	l1 same TWEEN	134	L11
USPT,JPAB,EPAB,DWPI	l9 and (sodium chloride or NaCl or calcium chloride or CaCl$1 or salt$3)	24	L10
USPT,JPAB,EPAB,DWPI	l6 and l1	28	L9
USPT,JPAB,EPAB,DWPI	(l6 or l7) and l1	112	L8
USPT,JPAB,EPAB,DWPI	TNBP	125	L7
USPT,JPAB,EPAB,DWPI	tri$1n$2butyl$1phosphate	175	L6
USPT,JPAB,EPAB,DWPI	alkyl$1phosphate$1free	0	L5
USPT,JPAB,EPAB,DWPI	alkyl$1phosphate$1free	0	L4
USPT,JPAB,EPAB,DWPI	alkyl phosphate$1free	0	L3
USPT,JPAB,EPAB,DWPI	alkyl phosphate$1free detergent	0	L2
USPT,JPAB,EPAB,DWPI	(virus or viral or microorganism or microbe or bacteria or bacterial or pyrogen$5) near5 inactivat$5	7496	L1

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USPT,JPAB,EPAB,DWPI	l1 and (sodium chloride or NaCl or calcium chloride or CaCl$1)	1	L5
USPT,JPAB,EPAB,DWPI	l1 and mM	2	L4
USPT,JPAB,EPAB,DWPI	chaotrophic	63	L3
USPT,JPAB,EPAB,DWPI	eluotroph$4	3	L2
USPT,JPAB,EPAB,DWPI	eluotrophic	3	L1

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L15: Entry 6 of 26 File: USPT Jun 16, 1998

DOCUMENT-IDENTIFIER: US 5766844 A
TITLE: Human T-cell line infected with HIV-2 which secretes functionally intact HIV-2 GP160.

DEPR:
The cell-free conditioned medium was used as the source of the HIV-2.sub.NIHz gp160. Usually 50 to 100 liter batches of the cells are grown in the serum-free medium in roller bottles. Cells were removed from the medium and sodium phosphate pH 7.5, sodium chloride, Triton X-100, and PMSF were added to the conditioned medium to final concentrations of 20 mM, 400 mM, 0.5w and 0.1 mM respectively in order to inactivate infectious virus. After incubation at room temperature for one hour the inactivated medium was concentrated approximately 50 fold using a Pellicon Cassette fitted with a 10K molecular weight cut-off filter (Millipore Corporation). The HIV-2.sub.NIHZ gp160 was purified from the inactivated and concentrated conditioned medium by immunoaffinity chromatography using a mouse monoclonal antibody to HIV-2 gp41. The monoclonal antibody was developed by ABL using partially purified HIV-2.sub.NIHZ gp160. The purified immunoglobulin of the monoclonal antibody was coupled to CNBr activated Sepharose (purchased from Pharmacia) at a concentration of 5 mg per ml according to the manufacturer's recommendations. The concentrated conditioned medium of 6D5.sub.NIHz (clone 11) cells was passed through a 50 ml column of the above antibody at 4.degree. C. After washing the column with phosphate buffered saline and then saline, the bound proteins from the column were eluted with 100 mM sodium carbonate containing 0.1 mM PMSF. The elution of the protein from the column was monitored by absorbance measurement at 280 nm using a LKB UVICORD connected to a recorder. The eluted protein was neutralized using 2N HCl, and sodium phosphate pH 7.5 was added to a final concentration of 10 mM. This fraction contained HIV-2.sub.NIHz gp160 along with human serum albumin (HSA) as a contaminant. To remove HSA, the sample was passed through a column of goat antibody to HSA (purchased from Cappel labs) coupled to Sepharose (5 mg per ml of Sepharose). The unbound fraction was concentrated to 10 to 20 ml and stored in suitable aliquots at -70.degree. C. FIG. 1 shows the SDS-PAGE profile of purified HIV-2.sub.NIHZ gp160. The purified protein was run in 7.5% SDS gels, stained with Comassie Blue, destained and photographed.

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Generate Collection

L15: Entry 19 of 26 File: USPT Jan 5, 1993

DOCUMENT-IDENTIFIER: US 5177194 A
TITLE: Process for purifying immune serum globulins

BSPR:
Prior to loading the immune serum globulin-containing liquid onto the column, the salt concentration of that liquid preferably is adjusted to an amount substantially equivalent to the salt concentration of the equilibration buffer. For example, if an acetate buffer is used for the acetate concentration in the immune serum globulin-containing liquid is adjusted to approximately the same concentration as that in the buffer. After loading the immune serum globulin-containing liquid onto the column, the column is advantageously washed sequentially with the same buffer used for equilibration. A preferred procedure involves employing sequential washes with decreasing concentrations of the virus-inactivating agents, with a final wash of at least ten times the bed volume of the column with a buffer devoid of the virus-inactivating agent. Sequential washes are advantageous in reducing resin-bound lipids while also removing the virus-inactivating agents from the cation exchange resin. Sequential washing has also been found to reduce pre-kallikrein activator, thus resulting in a final product substantially free of this protein. For example, after loading the column, it is washed with at least two times its bed volume with a 10 mM acetate buffer, pH 5.0-6.0, containing 1% Triton X-100 and 0.3% Tween 80, or a 10 mM acetate buffer, pH 5.0-6.0, containing 1% Triton X-100. This washing may be followed by washing with at least four times the column bed volume with a 10 mM acetate buffer, pH 5.0-6.0, containing 1% Tween 80 until the absorbance at 280 nM is less than about 1.2. When the A.sub.280 has decreased below 1.2, the column advantageously is washed with at least 20 times its bed volume with 10 mM acetate buffer, pH 5.0-6.0.

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L20: Entry 6 of 13 File: USPT Oct 5, 1999

DOCUMENT-IDENTIFIER: US 5962405 A
TITLE: Storage-stable fibrinogen preparations

BSPR:
EP-A-085 923 describes a lyophilized fibrinogen preparation which, aside from fibrinogen, further contains a substance which possesses an urea or a guanidine group. However, it has been demonstrated that lyophilized tissue adhesive preparations made accordingly act cytotoxically, inhibit the growth of fibroblasts and lead to an altered, nonphysiological fibrin structure whereby the desired elasticity of fibrin and the seal is lost (see Redl et al., Medizinische Welt 36, 769-76 (1985)). By the inhibition of the fibroblast growth, i.e. those cells which initiate the wound healing process, the desired wound healing promoting properties of tissue adhesives based on fibrinogen are lost. Further, the required high adhesive strength in vivo is jeopardized by the absent elasticity of the resulting fibrin.

DEPR:
A fibrinogen-containing human plasma fraction was treated with Tween 80 essentially as described in AU-B-18306/92 in order to inactivate viruses, such as HIV, which may be present in human plasma.

DEPR:
Thereafter, the material was dissolved at a protein concentration of 40g/l with a solution containing per liter 40 mmol histidine.HCl, 40 mmol niacinamide, 80 mg Tween 80, and 100,000 KIU aprotinin, adjusted to pH 7.3 with NaOH. Virus inactivated human albumin was added at a concentration of 6 g/l, and human purified Factor XIII, virus inactivated according to Austrian Patent Application A 1548/93, was added at a concentration of 15,000 U per liter.